PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 10-817-US)

In re Application of:	
John Crowley, et al.	Group Art Unit: 1653
Serial No: 10/591,118	Examiner: Barnhart, Lora Elizabeth
Filed: September 28, 2007	Confirmation No.: 7739
Title: Process for Cell Culturing by Continuous Perfusion and Alternating Tangential Flow	

DECLARATION PURSUANT TO 37 C.F.R. 1.132

I, Gerben M. Zijlstra, Ph.D., residing at Langewoldlaan 7, 9727 DE Groningen, The Netherlands, hereby declare:

1. I am currently a Senior Scientist at DSM Biologics, Groningen, The Netherlands ("DSM"). DSM IP assets is the assignee of the captioned application.
2. I have 22 years of experience in the field of cell culture technology, particularly in culturing animal and mammalian cell lines.
3. I hold a Ph.D. from the Agricultural University Wageningen in the Netherlands, which I received in 1998. The subject of my Ph.D. thesis was animal cell-culture in aqueous two-phase systems with the intention to retain animal cells in the bioreactor in one of the liquid phases, while extracting the product with the other liquid phase, thus integrating fermentation and (part of the) downstream processing in a single unit operation.
4. In 1994, I took a position at DSM as a Cell Culture Scientist in the Development Department, where, among my responsibilities, I was involved in several mammalian cell line development projects, mainly involving perfusion cell culture processes with cell retention devices including spinfilters and hollow fibers. I was also involved in setting up a laboratory for small scale pre-GMP culture, fermentation, and primary recovery of animal cells. I held this position from

1994-1997. From 1997-1999, I worked at DSM as a Senior Cell Culture Scientist, where I continued to work in cell culture.

5. From 1999-2001 I worked at DSM as a Scientist, Mammalian Cell Culture, in the Production Department. Representative projects include transfer of a insect cell - baculovirus perfusion process to DSM Montreal GMP facility, and testing of cell retention devices for a hybridoma development project.

6. In 2001 I was promoted to Senior Engineer at DSM, a position I held until 2004. In this role, I was responsible, among other things, for introducing new bioreactors, bioreactor controllers, and related equipment.

7. In 2005 I moved to my current position as Senior Scientist in the Process Development Department. My responsibilities in my current position include, among other things, the scale-up of cell culture processes and scouting, testing, and implementing new technologies in cell culture. In this position I have worked extensively with many cell retention devices amongst others ATF filtration units.

8. Based on my education and work experience as described above, I believe that I am of at least ordinary skill in the art of cell culture, particularly animal cell culture.

9. I have reviewed the specification for the captioned application, as well as the amended claims prepared for submission in response to the office action mailed July 7, 2011. I have also reviewed and considered the office action mailed July 7, 2011 and the prior art cited in the office action, specifically the Kyung reference, the Shevitz patent, and the Furey publication.

10. As one of skill in the art, I find claim 1, and its dependent claims, to be clear and unambiguous. In particular, I understand the scope of the claims, and would be able to assess whether a particular method of cell culturing infringes the claims.

11. In particular, I find nothing confusing or unclear in the phrase "wherein no more than 5% of the animal cells in the culture form aggregates of at least 5 cells during the continuous perfusion culturing" as it appears in claim 1. I am able to understand the scope of claim 1 (and its dependent claims) and how the recited limitation impacts the scope of the claims and whether or not a particular method would infringe claim 1 (and/or its dependent claims).

12. I also find nothing confusing or unclear in the phrase "resulting in an outflow of cell culture liquid through the pores of the filter module having a lower animal cell density per ml than the cell culture prior to circulating through the filter module"

as it appears in claim 1. I am able to understand the scope of claim 1 (and its dependent claims) and how this limitation impacts the scope of the claims and whether or not a particular method would infringe claim 1 (and/or its dependent claims).

13. I also find nothing confusing or unclear in the phrase "a lower animal cell density per ml" as it is used in claim 1. As one of skill in the art familiar with perfusion culturing, this phrase is clear and readily understandable to me. I am able to understand how this limitation impacts the scope of the claims and whether or not a particular method would infringe claim 1 (and/or its dependent claims).

14. I also find nothing confusing or unclear in the phrase "to compensate for the biomass removal" as it is used in claim 4. As one of skill in the art familiar with perfusion culturing, this phrase is clear and readily understandable to me. I am able to understand how this limitation impacts the scope of the claims and whether or not a particular method would infringe claim 1 (and/or its dependent claims).

15. Claims 8 and 9 provide additional limitations to claim 1 and, as with claim 1, I find these claims to be clear and unambiguous. In particular, I understand the scope of the claims, and would be able to assess whether a particular method of cell culturing infringes these claims.

16. As one of skill in the art, I do not find the claims to be obvious over the three cited references, either alone or in combination. Two elements of the method of claim 1 in particular represent a non-obvious advance over the prior art: (a) "wherein no more than 5% of the animal cells in the culture form aggregates in suspension of at least 5 cells"; and (b) "continuous perfusion culturing is continued until animal cells are present in the cell culture at a density of at least 80 x 10^6 viable animal cells/ml." Certainly, the extremely low aggregation of cells in suspension recited in the claims is not obvious in a cell culture with the recited high viable cell density.

17. A major focus of the Shevitz patent is on solving the problem of aggregate build up *at the filter* of the bioreactor, which causes clogging of the filter and, often, a failure of the bioreactor. At the time of the Shevitz patent, filter clogging was a significant problem in perfusion cell culture methods.

18. In fact, every reference in Shevitz to aggregation is in conjunction with build-up (and clogging) at the filter. For example, at Column 1, lines 54-57, Shevitz recites: "However, many of these **filters** have short operating lives, and when used to **filter** cell culture suspension or other biological fluids they **tend to clog with** dead cells, cell debris, **aggregates** or other constituents of the fluid" (emphasis added). There is no doubt to me that this reference to aggregates refers to aggregation and clogging at the filter.

19. Shevitz also mentions aggregation at Column 2, lines 54-56: "The resulting build up of dead cells and **aggregates on** screens or **filters, resulting in clogging** and failure of the perfusion device" (emphasis added). Again, there is no doubt that this passage refers to aggregation at the filters of the bioreactor, which causes failure of the bioreactor. This is precisely the aggregation problem that Shevitz sought to solve.

20. In another instance, Shevitz refers to aggregation at Column 3, lines 37-41: "Recirculation in one direction through the hollow fiber cartridge typically results in **clogging of the hollow fiber lumen by aggregates lodging at lumen inlet. Such aggregates may grow in size** and as more hollow fibers are blocked, filtration capacity declines" (emphasis added). Again, here Shevitz is referring to aggregation at the filter (hollow fiber cartridge).

21. In the final instance in which Shevitz refers to aggregation, Shevitz states at Column 14, line 64 through column 15, line 7: "The dynamics of the inventive system can extend the operating life of a perfusion run since pulsating flow between vessel 2 and chamber 30 greatly **inhibit the attachment of aggregates to the hollow fiber lumen or to the filter membrane**. For example, as culture medium flows from vessel 2 to pump 34, **aggregates** that are larger than the inside diameter of the hollow fibers **will be retained by the hollow fiber array; i.e., the hollow fiber will act as a filter,** however, by repeated and rapid reversal of flow direction, **the deposited aggregates are quickly removed and swept back to the vessel**" (emphasis added). In this passage, Shevitz discusses how the problem of aggregation at the filter is mitigated, such that the aggregates are not retained at the filter (hollow fiber array). The top of the filter acts as a coarse filter for large aggregates; once the large aggregates contact the top of the filter, they are removed and swept back into the vessel. This passage also makes it clear that aggregates are necessarily within the vessel

and will be sent back into the culture by the alternating flow without reducing aggregate size. Thus, Shevitz teaches that a solution to bioreactor failure due to filter clogging is elimination of the aggregate buildup at the filter.

22. The claimed invention, however, addresses a different problem, specifically a reduction in aggregation of cells *in suspension*. What is surprising about the claimed invention, and unexpected to one of skill in the art, is that the claimed methods are able to achieve a remarkably low level of aggregation – no more than 5% of the cells in culture form aggregates in suspension of at least 5 cells – in such a high density suspension – 80 x 10^6 cells/ml – of viable cells. This is counterintuitive, as one of skill in the art would expect an increase in aggregation in suspension as the cell density increases, and a significant level of aggregation at a high cell density. This, in fact, is exactly what Kyung shows, which I discuss in more detail below.

23. The Shevitz patent is silent on the reduction of viable cell aggregation in suspension, and may be viewed as implicitly accepting aggregation of cells in suspension, in focusing on minimizing the problems of other types of aggregation cause at the filter surface or inlet.

24. Kyung and/or Furey do not compensate for this deficiency. Kyung actually shows the difficulties faced in trying to reduce cell aggregation during cell culture. Kyung simply states that "293 cells form aggregates at high Ca^{2+} concentration," without stating what constitutes a "high Ca^{2+} concentration." Kyung then teaches the use of a cell culture medium comprising 100 μm Ca^{2+}, which was selected, presumably, to minimize cell aggregation, and follows by stating "[h]owever, over a prolonged cultivation period, some large aggregates did form." As I mentioned in paragraph 22 above, Kyung found a significant level of cell aggregation in suspension over a prolonged period of culturing, and at a high cell density – albeit at a cell density lower than the viable cell density recited in the currently pending claims (as discussed in more detail in paragraph 26). In particular, Figure 4 of Kyung shows micrographs of the aggregates found at two different time periods (120 or 125 hours (different times are given in the text on page 188 and in the figure legend on page 187) and 350 hours). These micrographs show very large clumps of cells that contain many more than 5 cells, and contain many dead cells. Kyung exemplifies exactly the problem of cell aggregation in suspension that the present invention overcomes.

25. The conclusion one of skill in the art reaches, in reading Kyung, is that cell aggregation in suspension will remain a problem even when efforts are made to mitigate it.

26. As one of skill in the art, I also believe that none of the three references, whether considered independently or together, makes obvious a method of limiting aggregation of suspended cells in cell culture where animal cells are present in the cell culture at a density of at least 80 x 10^6 viable animal cells/ml. Furey mentions the use of the Shevitz bioreactor for larger scale culturing and longer culture times (many days), but it doesn't discuss aggregation of suspended cells or the concentration of the cells or their viability – and it certainly does not teach or suggest a concentration of 80 x 10^6 viable animal cells/ml – or a concentration of 80 x 10^6 viable animal cells/ml, where no more than 5% of the animal cells in the culture form aggregates in suspension of at least 5 cells. Shevitz does not address concentration/viability either, other than to mention, for example at column 2, lines 54-56, that dead cells (non-viable cells) cause problems with clogging at the filter.

27. Unlike Furey and Shevitz, Kyung does discuss particular cell densities. Kyung teaches that at 400 hours of culture, a maximum of 9.3 X 10^7 cells/ml was achieved (page 188, left column, near end of first paragraph bridging pages 187 and 188). However, Kyung also states that at 400 hours cell viability was 85% (page 188, left column, first full paragraph, third line from end of paragraph). 85% of 9.3 X 10^7 cells/ml is 7.9 X 10^7 viable cells/ml. Thus, one of skill in the art would not have had a reasonable expectation of success that continuous perfusion culturing could be continued until animal cells are present in the cell culture at a density of at least 80 x 10^6 <u>viable</u> animal cells/ml, particularly where no more than 5% of the animal cells suspended in the culture comprise aggregates of at least 5 cells, as recited in the presently pending claims. Rather, Kyung shows a trend where a greater cell density results in lower cell viability (see page 188, first full paragraph, final sentences) and larger aggregates. With all of this taken together, one of skill in the art would not believe that Kyung's methods could be modified to achieve a *greater* density of viable cells, with *less* aggregation in suspension, as the present invention achieves. Rather, one of skill in the art would conclude the opposite, *i.e.* that greater cell densities lead to a significantly lowered level of cell viability.

28. In short, based on my understanding of the art and my conclusions based on the art, as discussed in paragraphs 16-27, I would not find the claimed invention obvious over the cited references.

29. Also, as I mentioned previously, the claimed invention provides results that would not be expected by one of skill in the art, that is, that the claimed methods are able to achieve a remarkably low level of aggregation – no more than 5% of the suspended cells in culture form aggregates of at least 5 cells – in such a high density suspension – 80 x 10^5 viable cells/ml. In particular, one of skill in the art would find this to be surprising because low shear conditions, such as in continuous perfusion cell culturing, typically do not lead to disaggregation of cells. Disaggregation of cells in suspension is a significant advance in perfusion cell culturing, because with cell aggregation, process control is more difficult, due to, for example, heterogeneity in metabolic profiles of cells within the cell aggregates. If no more than 5% of the cells form aggregates of at least 5 cells, such aggregates do not form a problem in cell culturing.

30. I hereby declare further that all statements made herein by me to my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

I, Gerben M. Zijlstra, Ph.D., declare under penalty of perjury that the foregoing is true and correct.

Dated: 31 OCT 11

Signed: _____
Gerben M. Zijlstra, Ph.D.